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A Novel Process for Removing Phosphate from Wastewater

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Introduction

Activated sludge plants are recognized as major point sources for phosphate (P) entering rivers and streams and causing eutrophication and toxigenic cyanobacterial blooms. Consequently, most plants now built are designed to remove P by a process called *Enhanced Biological Phosphate Removal* (EBPR). All are based on the principle that the biomass is repeatedly recycled through alternating anaerobic and aerobic stages (Seviour *et al*., 2003), a requirement considered essential for EBPR. Only then are the phosphate accumulating organisms (PAO) selectively advantaged, since in the anaerobic (FEED) stage these PAO rapidly assimilate substrates like acetate, which are used for synthesis of poly-β-hydroxybutyrate (PHB), and stored polyphosphate (polyP) is degraded to supply energy for its synthesis, with orthophosphate released into the medium. Then in the subsequent aerobic (FAMINE) stage, where exogenous substrates are scarce, the PAO can use their PHB stores to grow and assimilate orthophosphate to form polyp. The identity of the PAO, once believed to be gammaproteobacterial *Acinetobacter* spp are now thought from culture independent approaches, to be *Rhodocyclus* related bacteria (*Candidatus* 'Accumulibacter phosphatis' in the *Betaproteobacteria* Hesselmann *et al*., 1999; Crocetti *et al.,* 2000), with an ecophysiology of PAO (Kong *et al.,* 2004). Other organisms may also be important in EBPR (Kong *et al.,* 2005), and much still needs to be learned about EBPR microbial ecology (Seviour *et al.,* 2003).

However, globally not all existing plants operate as EBPR systems, and so the problems of P contamination remain. These produce effluents with low COD, but high P levels. One option would be to replace these with EBPR processes, but a cheaper strategy might be to design an 'add-on unit to an existing plant, dedicated to P removal. This talk describes such a novel aerobic process treating a synthetic 'effluent' containing 10-12 mg/l P and its microbial community.

The Process

A lab-scale SBR configuration was used, with a cycle time of 8 h, a hydraulic retention tome of

16 h and sludge age of 20 days. Stage 1 (***FAMINE***) allowed P uptake from P containing feed, stage 2 mixed liquor was removed, and in stage 3 (***FEED***) acetate containing mixed liquor was added, before stage 4, where biomass was allowed to settle and in stage 5 the now P depleted clarified liquor was removed. This process once stabilized (about 3 weeks) consistently gave < 0.5 mg/l P from and influent with 10-12 mg/L P, and in most cases no residual P could be detected.

Chemical Changes During SBR Cycle

During the **aerobic *FEED*** stage, acetate was rapidly assimilated and about 98% of it was used to synthesize PHB, an event corresponding to P release. With acetate exhaustion and P now available in the medium, PHB containing populations respired aerobically their stored PHB as carbon and energy source (as happens in the **anaerobic** stage of a conventional EBPR process). Intracellular PHB levels fell until all the released P had been reutilized, but then remained relatively unchanged when P had become exhausted from the medium. Then when P was added to the reactor (***FAMINE*** stage, corresponding to the **aerobic** stage of a conventional EBPR process), it was completely and rapidly assimilated by the P starved cells, probably utilizing PHB as an energy source. Biomass P content at the end of the ***FAMINE*** stage was 4.5%. Glycogen levels changed little over the cycle.

Microbial Ecology of this Aerobic Process

FISH analyses revealed that the *Betaproteobacteria* usually arranged in clusters, dominated the community, and that most of these (22.6% of the total cell biovolume) responded to the PAO mix and RHC probes targeting the same *Candidatus* 'Accumulibacter phosphatis' PAO seen in conventional EBPR processes. Combining FISH with DAPI staining showed that most cells (>85%) in samples taken at the end of the ***FAMINE*** stage which responded to these probes contained polyP. Most of the other clustered *Betaproteobacteria* (16.2% of total cells) fluoresced with the DECH454 probe designed in this study against *Dechloromonas* spp. None of these clustered cells stained positively with DAPI, suggesting they do not store polyP and thus are not PAO. *Alphaproteobacteria* were also common (7.9% of total cells), existing as tetrad forming organisms (TFO), and these responded to the DF988 FISH probe of Meyer et al (2006) targeting *Defluviicoccus* spp. Again none of these stained positively for polyP with DAPI.

Ecophysiology of Dominating Populations

Nile blue A staining in combination with FISH showed that the *Accumulibacter, Defluviicoccus* and *Dechloromonas* cells all fluoresced positively for PHB in samples taken during the FEED stage, although visually less strongly with *Accumulibacter* at the end of the FEED stages, suggesting this population

was reutilizing PHB during P reassimilation. Under conditions set up to mimic those existing in the SBR during the cycle, FISH/MAR showed that the *Accumulibacter* cells behaved as expected of PAO, assimilating ^{14}C- acetate during the ***FEED*** stage, and ^{33}P during the ***FAMINE*** stage. ^{14}C- acetate assimilation was also detected by both *Defluviicocus* and *Dechloromonas* in the ***FEED*** stage but neither showed an ability to assimilate ^{33}P during the famine stage

Discussion

This talk describes a novel aerobic process for P removal, where the selective pressures thought to operate in a conventional EBPR process were imposed in a fundamentally different way. Crucially important was the temporal separation of the additions to the reactor of the carbon/energy source acetate (***FEED*** stage) and the P containing low COD feed (***FAMINE*** stage). This cyclic aerobic process resulted in the enrichment of *Candidatus* 'Accumulibacter phosphatis' presumably because a) in the FEED stage, in the **absence of an external source of P**, these could utilize their polyP reserves to assimilate acetate into PHB and b) then in the ***FAMINE*** stage these P starved cells could rapidly and completely assimilate P supplied in a low COD feed into polyP using PHB as the energy source. Thus whereas conventional anaerobic:aerobic EBPR systems produce low P effluents from high COD feeds, this aerobic process produces a low P effluent from a low COD P feed. Interestingly the same PAO populations seem to be important in both. Both ***Dechloromonas*** **and** ***Defluviicocus,*** by being able to assimilate acetate in the ***FEED*** stage and synthesizing PHB, by not synthesizing polyP in the ***FAMINE*** stage appear to be potential competitors of the PAO, and have the phenotype of the GAO (Seviour et al 2003). Their impact on this process needs further examination, although glycogen biomass levels did not change markedly over the SBR cycle.

Finally, this study has shown that aerobic EBPR is achievable, and the oprocess described here is very reliable. Its future is not to replace conventional EBPR processes where P and COD are provided together, but as an add-on unit to remove P from low COD effluents (ie those generated by conventional activated sludge systems designed to remove carbon).

Acknowledgements

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